Mast cell stabilization potential of Sitopaladi churna: An ayurvedic formulation

Inder K. Makhija, Chandrashekara S. Shreedhara, H. N. Aswatha Ram

Department of Pharmacognosy, Manipal College of Pharmaceutical Sciences, Manipal University, Karnataka, India

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ABSTRACT

Background: Sitopaladi churna (SPC) is a popular polyherbal ayurvedic formulation used in the treatment of allergy and respiratory diseases. **Objective:** The present study was aimed to justify the classical use of antiallergic claim by performing the mast cell stabilizing activity of extracts of SPC. **Materials and Methods:** The protective effect of aqueous extract and methanolic extract - of SPC against compound 48/80-induced mast cell degranulation model was carried out. **Results:** Sitopaladi churna aqueous extract (SPCA) at the dose of 300 mg/kg and Sitopaladi churna methanolic extract (SPCM) at the doses of 150 and 300 mg/kg showed better protection of mast cell degranulation (65%-74%) and were comparable to the standard drug ketotifen (79%), when peritoneal mast cells were treated with compound 48/80. The protection against mast cell degranulation was significant ($P < 0.0001$). **Conclusion:** From the above results, it has been justified that SPC can be used to treat allergic disorders.

Key words: Compound 48/80, mast cell stabilizing, Sitopaladi churna

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INTRODUCTION

Mast cells are the constituents of virtually all organs and tissues and are important mediators of inflammatory responses such as allergy and anaphylaxis.[1] Sitopaladi churna (SPC) is a polyherbal ayurvedic medicine traditionally used as a supportive agent for allergy and in the treatment of cold, cough associated with bronchitis, pneumonia, and tuberculosis.[2] With this background, the study was carried out to justify the traditional claims by investigating its use in the treatment of allergy using a validated model.

MATERIALS AND METHODS

Chemicals

Compound 48/80, ketotifen fumarate and Roswell Park Memorial Institute (RPMI) 1640 medium was purchased from Sigma Aldrich, Bangalore, India. Toluidine blue was purchased from Sisco Research Laboratory, Mumbai, India. All other chemicals used were of analytical grade.

Address for correspondence:
Dr. H. N. Aswatha Ram, Department of Pharmacognosy, Manipal College of Pharmaceutical Sciences, Manipal University, Karnataka, India. E-mail: aswatharam@gmail.com

Plant material

SPC consists of five ingredients viz., *Saccharum officinarum* (sugar candy), Vamshalochana (siliceous concretion), *Piper longum* (dried fruit), *Elettaria cardamomum* (dried seed), and *Cinnamonum zeylanicumm* (stem bark). All these ingredients were procured from the local market of Udupi, Karnataka, India, and were authenticated by botanist Dr. Gopal Krishna Bhat, Professor, Department of Botany, Poorna Prajna College, Udupi, Karnataka, India. Voucher specimen of the same was deposited in the museum of Department of Pharmacognosy, Manipal College of Pharmaceutical Sciences, for future reference.

Preparation of SPC

The churna was prepared according to the procedure given in Ayurvedic Formulary of India. All the ingredients were powdered separately, passed through 80 # sieve and then mixed together in specified proportions to get uniformity blended churna.

Preparation of SPC extracts

SPCA was prepared by maceration of 100 g of the SPC in chloroform water for 7 days with intermittent shaking. The resulting extract was concentrated and lyophilized to obtain a brownish residue (yield 35.98% w/w). The SPCM was prepared by extracting SPC (100 g) exhaustively with methanol using a soxhlet apparatus for 48 h. The methanolic extract was concentrated under reduced

pressure at 40°C using a rotary evaporator and lyophilized at −40°C to obtain a reddish-brown syrupy residue (yield 28.34% w/w). Both the residues were stored in a dessicator until its use.

Animals

Healthy adult Wistar albino rats weighing about 200-250 g were used for the study. The animals were housed in polypropylene cages, maintained under the standard conditions. (12 h light: 12 h dark cycle; 25 ± 3°C; 35%-60% humidity). They were fed with standard rat pellet diet (Hindustan Lever Ltd., Mumbai, India) and water *ad libitum*. The Institutional Animal Ethical Committee of Kasturba Medical College, Manipal, India (IAEC/KMC/15/2010), approved the study.

Acute toxicity studies

Graded doses (50, 300, and 2000 mg/kg) of SPC were tested for oral acute toxicity studies according to Organization for Economic Co-operation and Development (OECD) 420 guidelines. In brief, after drug administration the animals were evaluated every 10 min for 4 h followed by 24, 48, and 72 h for any changes in spontaneous motor activity, gait, respiration, writhing, piloerection, and so on. Animals were followed up to 30 days for mortality.[3] Doses selected for further studies were between 1/60th, 1/20th, and 1/12th of the maximum tolerated dose.

Compound 48/80-induced allergy test

Compound 48/80-induced allergy test[4,5] was carried out using healthy adult albino rats. The rats were sensitized by subcutaneous injection of compound 48/80, 1 mg/kg body weight. The sensitized animals were divided into eight groups ($n = 6$). The first control group received 2% gum acacia solution (2 mL/kg, p.o.). The second group received standard drug ketotifen fumarate (1 mg/kg, p.o.). The other groups received different doses (50, 150, and 300 mg/kg) of SPCA and SPCM, respectively for 14 days. On the 14th day, 2 h after the assigned treatment, 10 mL of normal saline was injected into the peritoneal cavity of rats, after a gentle massage, the peritoneal fluid was collected and transferred into the siliconized test tubes containing 7-10 mL of RPMI 1640 medium (pH: 7.2-7.4). Purification of the peritoneal mast cells was done as explained by Percoll.[6] Crude peritoneal cell suspensions contained 3% mast cells, and the purity of the mast cells after gradient centrifugation was more than 90%. Purified mast cells (cell density of 2×10^6/mL) incubated with compound 48/80 (5 µg/mL) at 37°C for 10 min. Cells were stained metachromatically with toluidine blue (0.1% w/v, pH 1.0) and quantified by using a neubauer hemocytometer under a Olympus BX 41 microscope (magnification ×400). The viability of the mast cells was determined by their ability to exclude trypan blue. The trypan blue exclusion test indicated a viability of greater than 95%. The percentage of intact cells (granulated) and disrupted (degranulated cells) in different treated groups were calculated.

Statistical analysis

All results are expressed as mean ± SD, $n = 6$. Results were analyzed by one-way analysis of variance followed by Bonferroni's multiple comparison test to compare between control and test groups.

RESULTS

Acute toxicity studies

SPC extracts did not produce any death till 72 h at 2000 mg/kg, p.o. The animals in all the groups survived up to 30 days without any apparent adverse symptoms.

Compound 48/80-induced mast cell degranulation

SPCA and SPCM in the lower doses have showed least activity with $P < 0.01$ and $P < 0.001$, respectively. Whereas, SPCA at the dose of 300 mg/kg and SPCM at the doses of 150 and 300 mg/kg showed better protection of mast cell degranulation (65%-74%) was comparable to the standard drug ketotifen (79%), when peritoneal mast cells are treated with compound 48/80. The protection against mast cell degranulation was significant ($P < 0.0001$) as shown in Figure 1.

DISCUSSION

In type-I allergy, pathological mechanism involves the degranulation of mast cells, followed by the release of mediators such as histamine, leukotrienes, and prostaglandins from these cells.[7] The mast cells undergo degranulation in response to the immunological stimuli in which the antigen-antibody reaction on the cell surface predominates. Effect of SPC on mast cells degranulation was studied by administering the churna in rats is reported.[8] Antihistaminic and anti-inflammatory activity

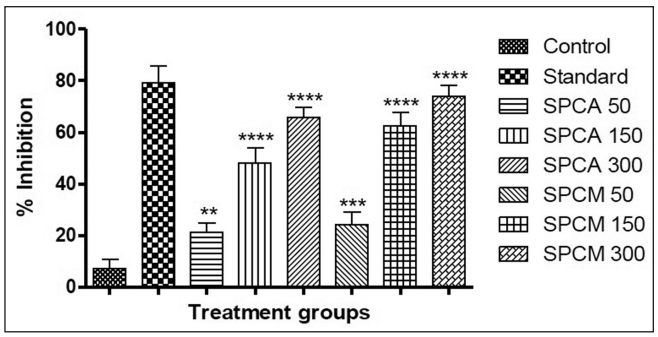

Figure 1: Effect of the SPC extracts on inhibition of mast cell degranulation in compound 48/80-induced degranulation in rats

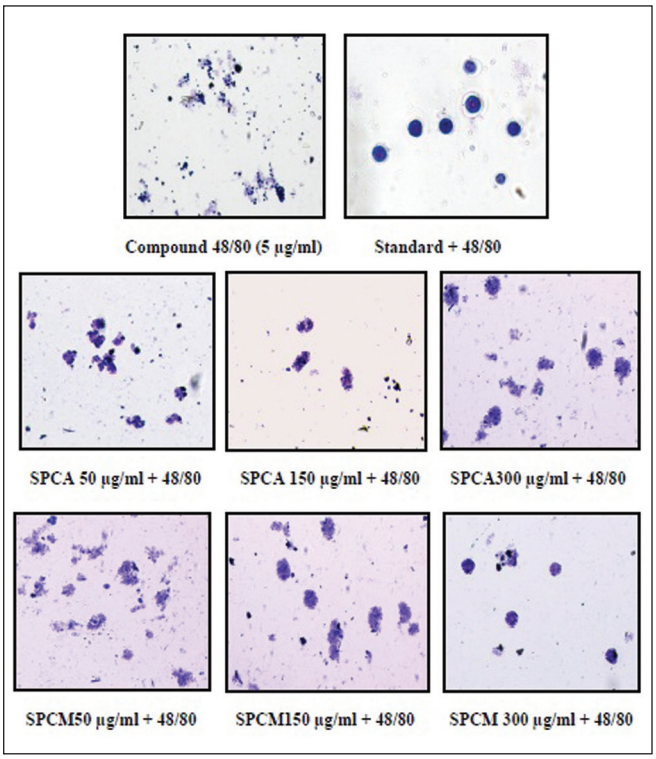

Figure 2: Microscopic photographs of rat peritoneal mast cells. Compound 48/80 (5 µg/mL), extensive degranulation processes (disrupted cells) and the presence of multiple granules extruded from the mast cells. Standard + 48/80, mast cell contains closely packed secretory granules (intact cells). Mast cells showed evident decrease of the degranulation in extracts treated in dose-dependent manner

of SPC extract in rats is also reported.[9] In the present investigation, the extracts of SPC have been studied and are active in the type-I allergic condition because of their ability to inhibit the release of mediators from mast cells [Figure 2] and thereby preventing the harmful effects of the released mediators. The mast cell stabilizing activity can be attributed to a great extent due to the presence of piperine of the formulation, as this principle has been reported to possess this activity.[10] The piperine content was found to be 9.51 ± 0.421 by high performance liquid chromatography. The preliminary phytochemical tests showed the presence of flavone glycosides in the extracts of SPC. Flavonoids have been reported to possess antihistaminic and antiallergic activity.[11-13] These results fully justify the use of SPC to treat allergic disorders. However, further studies are required to find the actual mechanism of antiallergic effects of constituents of churna.

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